The vagus regulates histamine mobilization from rat stomach ECL cells by controlling their sensitivity to gastrin

P. Norlén[1], P. Ericsson[2], M. Kitano[2], M. Ekelund[3] and R. Håkanson[2]

[1]*Department of Experimental and Clinical Pharmacology, Institute of Laboratory Medicine, Lund University Hospital, Lund, Sweden*
[2]*Department of Pharmacology, Institute of Physiological Sciences, University of Lund, Lund, Sweden*
[3]*Department of Surgery, Lund University Hospital, Lund, Sweden*

The ECL cells in the oxyntic mucosa secrete histamine in response to gastrin, stimulating parietal cells to produce acid. Do they also operate under nervous control? The present study examines histamine mobilization from rat stomach ECL cells *in situ* in response to acute vagal excitation and to food or gastrin following vagal or sympathetic denervation. Applying the technique of microdialysis, we monitored the release of histamine by radioimmunoassay. Microdialysis probes were placed in the submucosa on either side of the stomach, 3 days before experiments. The rats were awake during microdialysis except when subjected to electrical vagal stimulation. One-sided electrical vagal stimulation raised serum gastrin and mobilized gastric histamine. However, gastrin receptor blockade prevented the histamine mobilization, indicating that circulating gastrin accounts for the response. Vagal excitation by hypoglycaemia (insulin) or pylorus ligation did not mobilize either gastrin or histamine. The histamine response to food was almost abolished by gastrin receptor blockade, and it was halved on the denervated side after unilateral subdiaphragmatic vagotomy. While the histamine response to a near-maximally effective dose of gastrin was unaffected by vagotomy, the response to low gastrin doses was reduced significantly. Abdominal ganglionic sympathectomy failed to affect the histamine response to either food or gastrin. In conclusion, gastrin is responsible for most of the food-evoked mobilization of ECL-cell histamine. The histamine response to electrical vagal stimulation reflects the effect of circulating gastrin rather than a direct action of the vagus on the ECL cells. Vagal denervation was accompanied by an impaired histamine response to food intake, probably reflecting the right-ward shift of the serum gastrin concentration–histamine response curve. The results suggest that the vagus controls the sensitivity of the ECL cells to gastrin.

(Received 7 January 2005; accepted after revision 25 February 2005; first published online 3 March 2005)
Corresponding author P. Norlén: Department of Experimental and Clinical Pharmacology, Institute of Laboratory Medicine, Lund University Hospital, S-22185 Lund, Sweden. Email: per.norlen@skane.se

Histamine-secreting ECL cells are numerous in the oxyntic mucosa of the vertebrate stomach (Håkanson *et al.* 1986). They operate under the control of circulating gastrin (Håkanson *et al.* 1974, 1994). Histamine is mobilized from the ECL cells to stimulate acid secretion from adjacent parietal cells (Sandvik *et al.* 1987; Håkanson & Sundler, 1991; Waldum *et al.* 1991). In addition, both ECL cells and parietal cells are thought to operate under nervous control.

The oxyntic mucosa receives a rich supply of nerve fibres, running in the lamina propria along glands and blood vessels. These fibres have different origins and may derive either from 'extramural' (parasympathetic, sympathetic and sensory) neurones (extrinsic nerve supply) or from 'intramural' (enteric) neurones (intrinsic nerve supply). Extrinsic nerve fibres are relatively few, reaching the stomach wall via the vagus and the splanchnic, mesenteric and sacral nerves. The rich intrinsic nerve supply (the enteric nervous system) with its myenteric and submucosal ganglia is not only quantitatively prominent but also mainly responsible for controlling secretory activity and peristaltic activity. In the stomach wall the myenteric ganglia predominate over the submucosal ganglia (for a review see Gershon *et al.* 1994).

Neurones in the intramural ganglia contain different candidate neurotransmitters, including acetylcholine and various neuropeptides, amino acids and amines (Schultzberger *et al.* 1980; Ekblad *et al.* 1985, 1991; Green & Dockray, 1988; Furness *et al.* 1992; Hannibal *et al.* 1998). A large number of these candidate neurotransmitters have been screened for their effects on ECL-cell histamine

 DOI: 10.1113/jphysiol.2005.082677

mobilization *in vivo* after local infusion via a microdialysis probe placed in the submucosa of the stomach (Norlén *et al.* 2001). Gastrin and noradrenaline, and neuropeptides, such as vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP), were found to stimulate ECL-cell histamine mobilization whereas somatostatin and galanin were found to inhibit. These results are in accord with earlier observations on isolated ECL cells in primary culture (see Prinz *et al.* 1993; Sandor *et al.* 1996; Lindström *et al.* 1997; Zeng *et al.* 1998, 1999; Lindström & Håkanson, 2001). Together the findings suggest that the ECL cells express receptors for a variety of neurotransmitters, in support of the view that the ECL cells operate under nervous control. The observation that ECL cells respond to noradrenaline (and adrenaline) with histamine mobilization (Lawton *et al.* 1995; Lindström *et al.* 1997; Lindström & Håkanson, 2001; Norlén *et al.* 2001; Bernsand *et al.* 2003) suggests that sympathetic stimulation is capable of activating ECL cells *in situ*. Finally, there is experimental support for the view that the ECL cells operate under vagal control; with time unilateral vagotomy results in ECL-cell hypoplasia on the denervated side (Håkanson *et al.* 1984, 1992; Axelson *et al.* 1988), suggesting a tonic influence of the vagus on the ECL-cell population.

The present study examines (1) the mobilization of histamine from rat stomach ECL cells in response to acute vagal excitation and (2) their response to food or gastrin following vagal or sympathetic denervation.

Methods

Drugs

Human Leu^{15}-gastrin-17 was obtained from Research Plus (South Plainfield, NJ, USA) and dissolved in 0.9% NaCl, containing 1% bovine serum albumin (Sigma). It differs from natural gastrin-17 in that the methionine residue in position 15 is replaced by leucine. The substitution of leucine in this position does not affect the biological activity (Morley & Smith, 1968) but prolongs shelf-life: the oxidized form of natural gastrin (i.e. the methionine sulfoxide analogue) is inactive or weakly active (Morley *et al.* 1965). Gastrin was given by intravenous infusion at a concentration of 5 nmol kg^{-1} h^{-1} for 4 h. Human insulin (Actrapid, Novo Nordisk, Bagsvaerd. Denmark) was injected subcutaneously at a dose known to induce vagal excitation and acid secretion without affecting the serum gastrin concentration (0.6 IU kg^{-1}, Ekelund *et al.* 1982). The gastrin receptor antagonist YF476 [(3R)-*N*-[1-*tert*-butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1H-1,4-benzodiazepine-3-yl]-N^1-(3-(methylamino)phenyl) urea] was obtained from Dr A. Harris (Ferring, Copenhagen, Denmark) and emulsified in polyethylene glycol 300 (Acros Organics, Geel, Belgium). It was given as a single subcutaneous injection 24 h before start of the experiments (300 μmol kg^{-1}). This dose regimen is known to induce a sustained near-maximally effective gastrin receptor blockade (Kitano *et al.* 2000*a*).

Animals

Male Sprague-Dawley rats (250–300 g) were kept at a 12-h light and 12-h dark cycle in plastic cages (2–3 in each cage) with free access to standard rat food pellets (B & K Universal, Sollentuna, Sweden) and tap water. All rats were fasted for at least 24 h before the experiments. During fasting they were housed in individual cages with wire mesh bottoms and deprived of food but with free access to water. In experiments involving refeeding they were offered standard food pellets and water for 4 h. Surgery involved pylorus ligation, dorsal or ventral unilateral subdiaphragmatic vagal denervation, upper abdominal sympathectomy, and implantation of microdialysis probes and jugular vein catheters (for details see below). Because of the inhibitory effects of anaesthesia on ECL-cell histamine mobilization (Norlén *et al.* 2000; Cui *et al.* 2002), microdialysis experiments were performed on conscious animals, except those experiments that involved electrical vagal stimulation (see below). During the experiments, the rats were kept in Bollman-type restraining cages. Starting 1 week prior to the experiments all rats were thoroughly familiarized with such cages by daily training for 1–2 h. Blood samples for determination of serum gastrin were drawn from the tip of the tail during the equilibration period and at the conclusion of the experiment. After the experiments each rat was killed by an intraperitoneal overdose of chloral hydrate. The studies were approved by the local Animal Welfare Committee, Lund/Malmö.

Surgery

Surgery was performed on rats anaesthetized with chloral hydrate (300 mg kg^{-1}, given as a single intraperitoneal bolus dose). Clean (but not sterile) instruments were used. Antibiotics were not used. Buprenorphine (Temgesic, Schering-Plough, NJ, USA) was given subcutaneously (0.05 mg kg^{-1}) after surgery to alleviate postoperative pain in rats subjected to sympathectomy. Supplemental doses were given after 6 and 16 h. In other experiments the short time interval between surgery and experiments precluded the use of buprenorphine, since opioids are thought to operate in the enteric nervous system of the stomach (as candidate transmitters) (Nishimura *et al.* 1984; Ekblad *et al.* 1991; Sternini *et al.* 2004) and to affect ECL-cell histamine mobilization (Norlén *et al.* 2001), and since buprenorphine is known to induce gastrointestinal distress in rats (Clark *et al.* 1997; Thompson *et al.* 2004). No mortality was associated with the surgery.

Gastric submucosal microdialysis. Implantation of the microdialysis probe was performed as previously described (Kitano *et al.* 2000*b*; Ericsson *et al.* 2003). The microdialysis probes (MAB3.8.10, AgnTho's AB, Stockholm, Sweden) had the following specifications: length 10 mm, outer diameter 0.57 mm, 35 kDa cut-off. Briefly, the abdomen was opened by a midline incision, and the ventral and/or the dorsal aspect of the stomach wall (acid-producing part) was tangentially punctured by a needle, creating a tunnel in the submucosal layer. The probe was gently inserted into the tunnel and the inlet and outlet tubes of the probe were passed through the abdominal opening and tunnelled under the skin to a point at the nape of the neck. At the same time, rats were fitted with a catheter in the right jugular vein (for details see Kitano *et al.* 2000*b*; Ericsson *et al.* 2003).

Pylorus ligation. Rats were equipped with a microdialysis probe (dorsal side of the stomach). At the same time, a loose-fitting loop (noose) of silk thread (4-0) was placed around the pylorus. Both ends were passed through a plastic catheter that was tunnelled under the skin to the neck. In this way, the two ends could be easily accessed at the time of experiment (3 days later) without disturbing the rat. The noose around the pylorus was tightened by traction (no anaesthesia). The ligation was maintained until the animals were killed 4 h later (the tightness of the ligation was verified at necropsy). The gastric juice was collected and its volume determined.

Unilateral vagotomy. The effect of unilateral vagotomy was studied on rats that had been subjected to section of either the anterior or the posterior vagus trunk under the diaphragm close to the stomach. Microdialysis probes were implanted on both sides of the stomach in the same surgical session.

Upper abdominal sympathectomy. The coeliac and superior mesenteric ganglia were extirpated. The operation was performed as described by Holmes *et al.* (1967) by the aid of an operation microscope. Briefly, the ganglia were excised by removal of tissue between the ventral surface of the aorta and the pancreas (between the coeliac and superior mesenteric arteries). The aorta was freed from surrounding fat and connective tissue, which also was excised. Excised tissue was fixed in 4% formaldehyde and examined for the presence of ganglionic cells by routine histology (haematoxylin–eosin staining) to verify success of surgery. Another group of rats were subjected to sham operation (laparotomy). All rats were left to recover for 2 weeks before implantation of microdialysis probes on the dorsal side of the stomach.

Experimental design

The rats were subjected to experiments 3 days after implantation of the microdialysis probes, at which time they had been fasted for 24 h. Unless otherwise stated, rats were awake during microdialysis and kept in Bollman-type restraining cages. Each rat and each probe were used once only. The inlet tube of the microdialysis probe was connected to a microinfusion pump (Model 361, Sage Instrument, ATI Orion, Boston, MA, USA) and the outlet was allowed to drain into 300 μl polyethylene vials. Before the experiments started the microdialysis probes were perfused with saline (1.2 μl min^{-1}) for 2 h to ensure stable baseline histamine levels (Ericsson *et al.* 2003). Microdialysate samples were collected hourly during a 2 h basal period and during a 3–4 h stimulation period, except during the first hour of stimulation when samples were collected every 10 or 20 min. Blood samples (200 μl) for determination of gastrin were collected from the tip of the tail once during the equilibration period and once after collecting the last microdialysate sample if not otherwise stated.

Vagal stimulation. (1) *Electrical stimulation.* Rats to be subjected to electrical vagal stimulation were equipped with microdialysis probes on either the ventral side (1 and 20 Hz) or on both sides of the stomach (5 Hz), 3 days before the experiments. During the experiments they were kept on a warm surface (37°C) under fluanisone : fentanyl : midazolam (15 : 0.5 : 7.5 mg kg^{-1}) anaesthesia. The anaesthetic cocktail was given by intraperitoneal injection. The depth of anaesthesia was monitored continuously by testing eyelid reflexes. When rats responded to stimulation, an additional 30% of the original dose was given by intraperitoneal injection. After an equilibration period of 1 h the abdominal wall was opened and the ventral vagus gently exposed along the oesophagus. After another 15 min, basal microdialysate samples were collected every 30 min for 1 h. The intact ventral vagus nerve was stimulated electrically by means of a pair of platinum electrodes (diameter 0.25 mm, distance between electrodes 2 mm). A Grass stimulator (S48 stimulator, Astro-Medical, W Warwick, RI, USA) generated 1 ms impulses of 5 V at 1 Hz (5 rats), 5 Hz (17 rats) or 20 Hz (7 rats). Microdialysate samples were collected every 10 min for 60 min if not otherwise stated. Five of the rats stimulated at 5 Hz had been pretreated with YF476 (as above). In these rats microdialysate was collected every 10 min for 40 min. Blood samples were collected from the tip of the tail 30 min before and 15 min after starting the electrical vagal stimulation.
(2) *Insulin-induced hypoglycaemia.* After 2 h basal sampling, insulin (0.6 IU kg^{-1}, Ekelund *et al.* 1982) was given by a subcutaneous bolus injection (8 rats). Microdialysate samples were collected hourly except

Table 1. Serum gastrin concentration in response to refeeding and to vagal stimulation by insulin injection or pylorus ligation

	Serum gastrin (pmol l^{-1})		
Refeeding	Preprandial	Postprandial	No. of rats
Sham operation	16 ± 1.5	41 ± 7.2**	8
Unilateral vagotomy	19 ± 2.5	45 ± 5.9**	8
Sympathectomy	32 ± 3.4	46 ± 4.6*	5
Vagal stimulation	Before stimulation	During stimulation	
Insulin injection	17 ± 1.5	21 ± 1.9 ns	8
Pylorus ligation	20 ± 1.7	28 ± 3.6 ns	6

*$P < 0.05$,**$P < 0.01$; ns, not significant.

during the first hour after insulin injection when samples were collected every 20 min.

(3) *Pylorus ligation.* Vagal stimulation was induced by acute pylorus ligation (6 rats). The ligation was maintained throughout the experiment (4 h). Microdialysate was collected immediately before and during the 4 h stimulation period.

Refeeding. After basal sampling of microdialysate for 2 h, seven fasted rats were given free access to food (and water). The effect of refeeding was also assessed in rats subjected to unilateral (ventral or dorsal) vagotomy ($n = 21$). One group of the vagotomized rats (8 rats) was fasted and refed following pretreatment with the gastrin receptor antagonist YF476. They had all been fitted with microdialysis probes on both the intact and the vagotomized side of the stomach. In addition, the effect of refeeding was assessed in rats subjected to surgical sympathectomy (5 rats).

Gastrin infusion. Rats fitted with microdialysis probes and with a catheter in the right jugular vein were fasted and then given an intravenous infusion of synthetic human Leu^{15}-gastrin-17 for 3 h (0.015, 0.05, 0.15, 0.7 and 5 nmol kg^{-1} h^{-1}). The infusion started after a 2 h basal period. The effect of gastrin infusion was assessed in rats that had been unilaterally vagotomized ($n = 28$, bilateral probes) or in rats that had been subjected to surgical sympathectomy (5 rats).

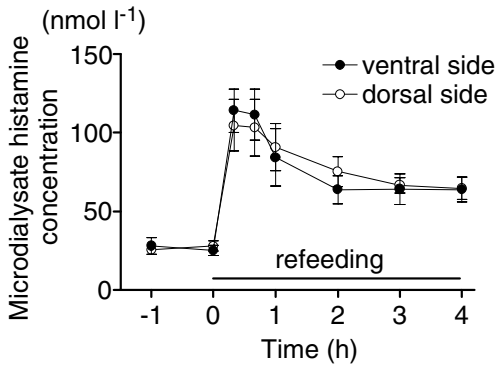

Figure 1. Effect of refeeding on histamine mobilization from the ventral and the dorsal side of the stomach in fasted rats
The rats were fitted with microdialysis probes on both sides of the stomach. After 24 h of food deprivation they received standard rat chow (time zero). Access to food is indicated by straight line. Microdialysate samples were collected simultaneously from the two sides of the stomach. Mean values ± S.E.M. ($n = 7$).

Biochemical analyses

Determination of gastrin. Serum gastrin was determined with a radioimmunoassay using antiserum 2604 (a kind gift from Professor J. F. Rehfeld, Copenhagen, Denmark) with rat gastrin-17 as standard and monoiodinated ^{125}I-gastrin-17 as tracer. Antiserum 2604 was raised against the 2–17 fragment of human gastrin-17 and is specific for the bioactive C-terminus. It binds gastrin-34 and gastrin-17 with the same potency. The gastrin concentration was expressed as picomole equivalents of rat gastrin-17 per litre.

Determination of histamine. Histamine in the microdialysates was measured by radioimmunoassay using a commercially available kit (Immunotech, Marseilles, France). The histamine concentration was expressed as nanomoles per litre.

Statistical analysis

All values are expressed as mean ± standard error of the mean (S.E.M.) or as the 95% confidence interval (CI) of the mean. Statistical significance was assessed using Student's *t* test, or by one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test ($P < 0.05$ was considered significant). Dose–response curves, concentration–response curves, EC_{50} values (the concentration producing 50% of the maximal effect), and the CI were constructed/calculated using the GraphPad PRISM program (version 3.00, GraphPad Software, San Diego, CA, USA).

Results

Histamine mobilization in response to food intake and vagal excitation

Food intake. Refeeding after a period of food deprivation resulted in a 2- to 3-fold increase in the serum gastrin concentration (Table 1). Also the microdialysate histamine concentration increased rapidly and peaked within 20–40 min (almost 4-fold increase). The peak was followed by a gradual decline to a level that was still more than 2-fold higher than basal after 4 h (Fig. 1).

Vagal stimulation. (1) *Electrical stimulation.* Electrical stimulation of the ventral vagus at 1 Hz was without effect whereas stimulation at 5 and 20 Hz raised the serum gastrin concentration 4- and 2-fold, respectively (Fig. 2*A*).

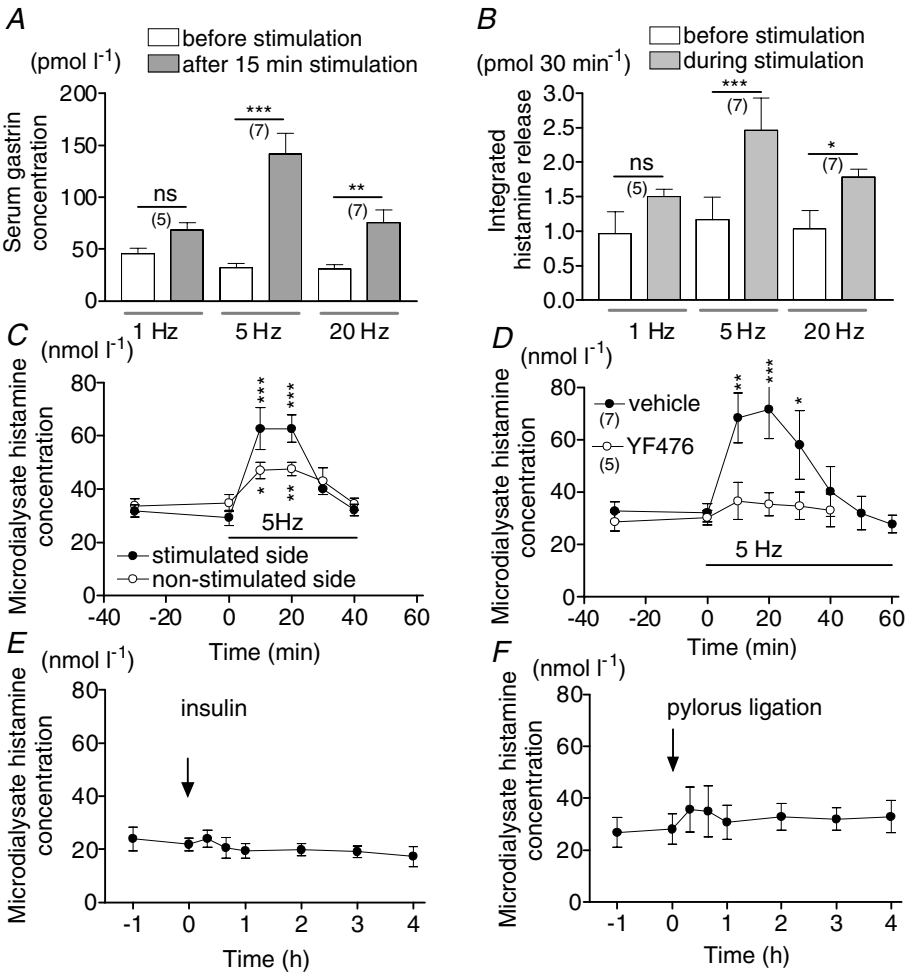

Figure 2. Effect of vagal stimulation on gastric histamine mobilization
In *A* and *B*, the ventral vagus nerve of fasted rats was exposed below the diaphragm and stimulated electrically with 1 ms impulses of 5 V at 1, 5 or 20 Hz. In *A*, the serum gastrin concentration was determined 30 min before and 15 min after start of electrical stimulation (blood sampling from the tail). In *B*, the microdialysate histamine output during 30 min of electrical stimulation was integrated and compared with the integrated output during 30 min before stimulation. In *C*, a histamine response was noted on both the stimulated (asterisks above the curve) and the non-stimulated side (asterisks below the curve) of the stomach in response to electrical stimulation (5 Hz, horizontal line) of the ventral vagus nerve ($n = 5$). The integrated histamine response (pmol (30 min)$^{-1}$) (not shown) was 2-fold greater on the stimulated than on the non-stimulated side ($P < 0.05$). In *D*, it is shown that pretreatment with the gastrin receptor antagonist YF476 (300 μmol kg^{-1}, administered as a single subcutaneous dose 24 h before the experiments) virtually abolished the response to electrical stimulation (horizontal line) (results from vehicle-treated rats are given for comparison). Finally, the effects of insulin injection (*E*) and pylorus ligation (*F*) on the microdialysate histamine concentration in fasted rats were assessed. Insulin was given as a single subcutaneous injection (0.6 IU kg^{-1}) at time zero (arrow) ($n = 8$). The pylorus was ligated at time zero (arrow): the ligation was maintained for 4 h (experiment terminated) ($n = 6$). Neither insulin injection nor pylorus ligation mobilized histamine. Mean values ± S.E.M. Statistical significance was assessed by ANOVA, followed by Dunnett's multiple comparison test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

On the whole, the amounts of histamine mobilized in response to vagal stimulation at different frequencies increased in parallel to the increase in the serum gastrin concentration (Fig. 2*B*). It is notable that unilateral vagal stimulation mobilized histamine from the non-stimulated as well as the stimulated side of the stomach (Fig. 2*C*). The integrated histamine response over 30 min was 2-fold higher on the stimulated than on the non-stimulated side (1.29 ± 0.23 pmol *versus* $0.60 \pm 0.0.21$ pmol, $P < 0.05$). The histamine response to vagal stimulation was virtually abolished after pretreatment with the gastrin receptor antagonist YF476 (Fig. 2*D*).

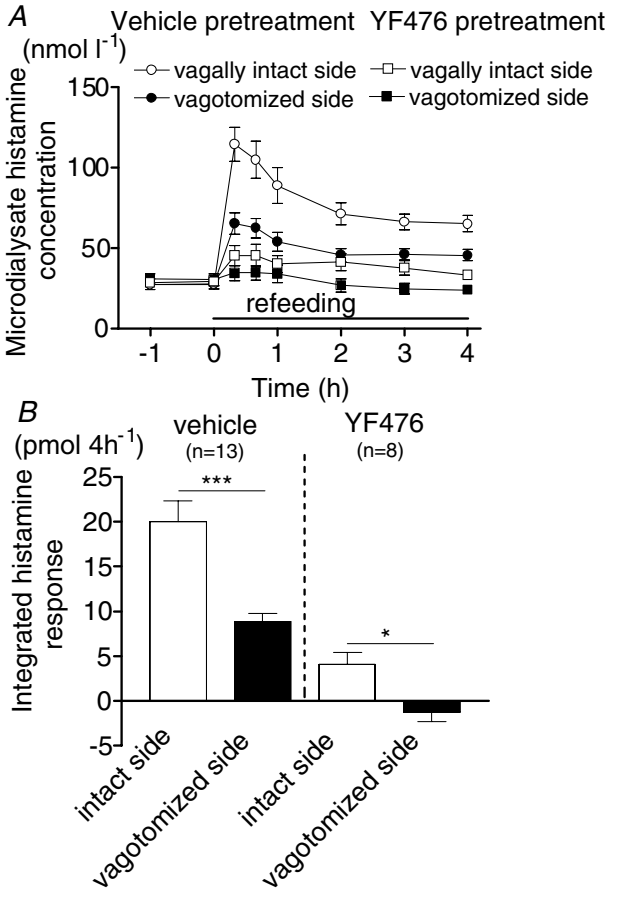

Figure 3. Effect of refeeding on gastric histamine mobilization in fasted rats

Unilateral (ventral or dorsal) vagotomy had been performed 3 days before microdialysis (microdialysis probes on both sides of the stomach). One group of vagotomized rats received a maximally effective dose of the gastrin receptor antagonist YF476 (300 μmol kg^{-1}, as a single subcutaneous injection, Kitano *et al.* 2000*a*) 24 h before the experiments (*A*). Rats were given standard rat chow at time zero (refeeding indicated by horizontal line) and microdialysate samples were collected simultaneously from the ventral side and the dorsal side. In *B*, the histamine response to refeeding (4 h) is integrated from data in *A*. Mean values ± S.E.M. The number of rats is indicated. Statistical significance was assessed by ANOVA followed by Dunnett's multiple comparison test (*$P < 0.05$, ***$P < 0.001$).

(2) *Insulin.* A subcutaneous injection of insulin (known to cause vagal excitation and to stimulate gastric acid secretion through hypoglycaemia, Ekelund *et al.* 1982) failed to mobilize ECL-cell histamine (Fig. 2*E*); the serum gastrin concentration was not affected by insulin (Table 1). (3) *Pylorus ligation.* Tying a ligature around the pylorus induced copious secretion of gastric juice; after 4 h the stomachs contained 11 ± 2.0 ml (as compared with 0.8 ± 0.5 ml in sham-operated rats). This is thought to reflect vagal excitation (Håkanson *et al.* 1980; Alumets *et al.* 1982). Pylorus ligation failed to mobilize either gastrin (Table 1) or ECL-cell histamine (Fig. 2*F*).

Histamine mobilization following vagotomy or sympathectomy

Unilateral vagotomy. A series of experiments was carried out on rats that had been unilaterally vagotomized. The histamine response to food intake was reduced by 56% on the denervated side (Fig. 3*A* and *B*). There was no difference between ventral and dorsal vagotomy in this respect (not shown). YF476 pretreatment reduced the food-evoked histamine mobilization by 80% on the intact side (compared with vagally intact, untreated control rats) and abolished the response on the vagotomized side (Fig. 3*A* and *B*).

Histamine mobilization in response to a near-maximal dose of gastrin (5 nmol kg^{-1} h^{-1}, circulating gastrin concentration 1480 ± 240 pmol l^{-1}) did not differ between the intact and the vagotomized side of the stomach (Fig. 4*A* and *B*). However, at submaximally effective gastrin doses, resulting in serum gastrin concentrations between 30 and 550 pmol l^{-1}, there was a reduced histamine response on the vagotomized side of the stomach (58% reduction, $P = 0.016$) (Fig. 4*C*). This presumably reflects a rightward shift of the serum gastrin concentration–histamine response curve following unilateral vagotomy: the EC_{50} value on the intact side was 67 pmol l^{-1} (95%CI, 6–730 pmol l^{-1}), while the EC_{50} value on the vagotomized side was 470 pmol l^{-1} (95% CI, 90–2400 pmol l^{-1})) (Fig. 4*C*).

Surgical (abdominal) sympathectomy. Food intake was associated with a 3-fold increase in microdialysate histamine in both intact and sympathectomized rats; there was no difference between the groups (Fig. 5*A*). Gastrin infusion for 3 h resulted in a 4-fold increase in microdialysate histamine in both sympathectomized and intact rats (Fig. 5*B*).

Discussion

Histamine mobilization in response to gastrin and to food intake

Gastrin is a powerful stimulator of ECL cells *in situ* (Håkanson *et al.* 1974, 1994; Kitano *et al.* 2000*b*). As

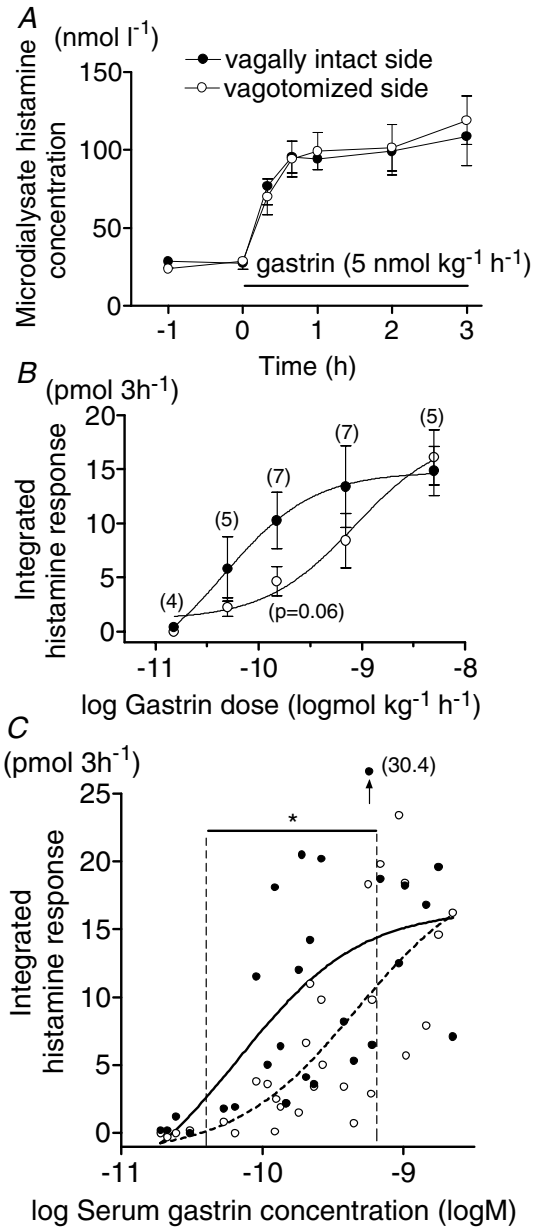

Figure 4 Effect of vagotomy on gastric histamine mobilization
A, gastric histamine mobilization in response to an intravenous infusion of gastrin (5 nmol kg^{-1} h^{-1}) in unilaterally vagotomized rats. Ventral vagotomy was performed on 5 rats 3 days before the experiments. Gastrin infusion (3 h) is indicated by horizontal line. Mean values ± S.E.M. The histamine response (integrated over 3 h) to different doses of gastrin (given by intravenous infusion) (*B*) and to the resulting serum gastrin concentrations (*C*) was assessed in 28 rats subjected to ventral vagotomy. Microdialysate samples were collected simultaneously from both the ventral (vagally denervated) and the dorsal (vagally intact) side in all rats. The gastrin dose–response and concentration–response curves for the vagally intact side and the vagally denervated side were compared. In *B*, each point is the mean of 4–7 rats. Each rat received one dose of gastrin only. Bars give S.E.M. The *P* value for the difference between the histamine response of the vagally intact and the denervated side at a gastrin dose of 0.15 nmol kg^{-1} h^{-1} was 0.06. Integrated values for the histamine output *versus* the serum gastrin concentration are given for each individual rat in *C*: ●, the histamine output on the vagally intact side; ○, the output on the vagotomized side of the stomach. The two sides were compared within the serum gastrin concentration range of 40–550 pmol l^{-1} (indicated by vertical lines). The statistical significance of the difference between the two sides was assessed by ANOVA followed by Dunnett's multiple comparison test (*B*) or by Student's paired *t* test (*C*). $^*P < 0.05$. Dose–response and concentration–response curves were constructed by the GraphPad PRISM program. In *C*, the drawn curve represents the intact side while the dotted curve represents the denervated side.

expected, it was found to mobilize ECL-cell histamine equally well on either side of the stomach (Fig. 1).

Food intake is known to cause vagal excitation and gastrin release. This is followed by stimulation of the ECL cells. Since gastrin receptor blockade greatly reduced the histamine response of the ECL cells to food (−80%, Fig. 3) (see also Kitano *et al.* 2000*b*), we propose that gastrin is the major stimulus behind food-evoked activation of histamine mobilization from the ECL cells. The question addressed in this study is whether the vagus and the sympathetic nervous system *contribute* to the food- and gastrin-evoked responses of ECL cells *in situ*.

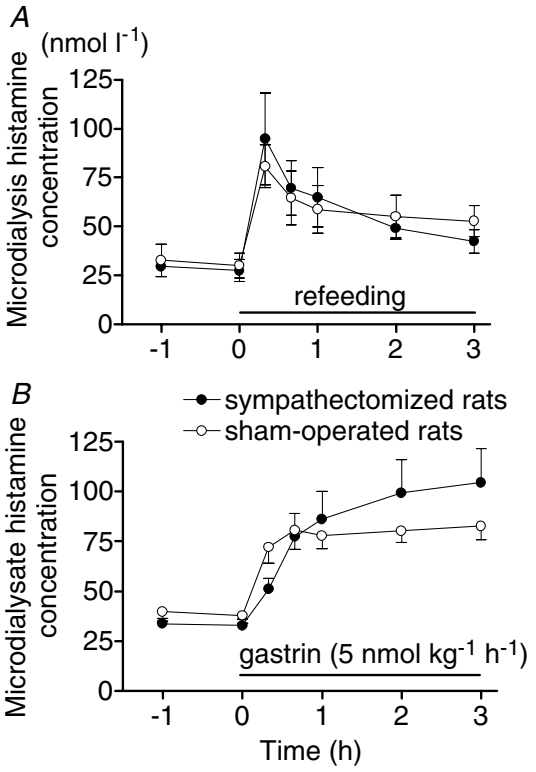

Figure 5. Effect of surgical sympathectomy on food-evoked (*A*) and gastrin-evoked (*B*) gastric histamine mobilization in fasted rats
Duration of refeeding or intravenous gastrin infusion (5 nmol kg^{-1} h^{-1}) is indicated by horizontal lines. Mean values ± S.E.M. ($n = 5$).

Histamine mobilization in response to vagus stimulation

Food intake is thought to stimulate gastric acid secretion not only via activation of the gastrin-ECL cell–parietal cell axis but also via vagal excitation (of the ECL cells or the parietal cells or both). In the rat, the importance of the vagus is illustrated by the fact that vagal excitation induced by pylorus ligation or insulin hypoglycaemia stimulates acid secretion (Brodie & Knapp, 1966; Håkanson *et al.* 1980; Ekelund *et al.* 1982), and that vagal denervation inhibits both basal and stimulated acid secretion (Håkanson *et al.* 1982; Vallgren *et al.* 1983). It is traditionally argued that vagal excitation stimulates the parietal cell through cholinergic mechanisms since acid secretion can be inhibited by atropine (and pirenzepine) (Ekelund *et al.* 1987; Riedel *et al.* 1988). Paradoxically, however, cholinergic agents, such as carbachol and bethanechol, are poor secretagogues in the chronic gastric fistula rat (Ding & Håkanson, 1996; Nishida *et al.* 1996). Although the ECL cells do not seem to operate under cholinergic control (Lindström *et al.* 1997; Lindström & Håkanson, 2001; Norlén *et al.* 2001), they do respond with secretory activation to a number of neuropeptides present in neurones in the stomach wall, e.g. pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) (Lindström *et al.* 1997; Zeng *et al.* 1998, 1999; Lindström & Håkanson, 2001; Norlén *et al.* 2001). In addition, they respond to adrenaline and noradrenaline (Lawton *et al.* 1995; Lindström *et al.* 1997; Lindström & Håkanson, 2001; Norlén *et al.* 2001; Bernsand *et al.* 2003). To study the importance of the nervous input for the functional activity of the ECL cells, we decided to examine ECL-cell histamine mobilization in response (1) to vagal stimulation and (2) to food and gastrin following surgical ablation of the vagal and sympathetic inputs.

Electrical vagal excitation elicited gastrin release (Fig. 2*A*) (see also Schubert *et al.* 1982; Alino *et al.* 1983) unlike pylorus ligation and insulin hypoglycaemia (Table 1) (see also Ekelund *et al.* 1982; Zhao *et al.* 1996). The lack of effect of pylorus ligation and insulin hypoglycaemia on circulating gastrin may reflect the dual effect of the vagus: it both stimulates and inhibits gastrin release (see e.g. Debas *et al.* 1984). The gastrin response to insulin is dose dependent. The dose employed in the present study is known to cause hypoglycaemia and acid secretion but not gastrin release (Ekelund *et al.* 1982). In the present study, electrical vagal stimulation (5 Hz) increased the microdialysate histamine concentration: it peaked within 10–20 min (2-fold increase) and then declined to prestimulation values. The histamine response was preceded by a 4-fold increase in the serum gastrin concentration (Fig. 2*A* and *B*). Such an increase most likely stimulates ECL-cell histamine mobilization (see e.g. Konagaya *et al.* 2001). It is not surprising therefore that one-sided electrical vagal stimulation resulted in histamine mobilization on both sides of the stomach (albeit more effectively on the stimulated than on the non-stimulated side) (Fig. 2*C*), and that gastrin receptor blockade (YF476) virtually abolished the histamine response (Fig. 2*D*). Thus, it seems that electrical vagal stimulation mobilizes ECL-cell histamine via release of gastrin rather than via a direct action on the ECL cells. Neither pylorus ligation nor insulin injection mobilized ECL-cell histamine (Fig. 2*E* and *F*) (or gastrin), which is in agreement with an earlier report demonstrating the lack of effect of pylorus ligation on the ECL-cell histidine decarboxylase activity (Zhao *et al.* 1996).

Histamine mobilization following vagal denervation

We monitored food- and gastrin-evoked ECL-cell histamine mobilization after unilateral vagotomy, taking advantage of the fact that each vagal trunk innervates one side of the stomach only and that denervation of one side does not impair the functional capacity of the other (Håkanson *et al.* 1984, 1992). By placing microdialysis probes on either side of the stomach, histamine mobilization on the intact side of the stomach can be compared with that on the vagotomized side. Unilateral vagotomy, performed 3 days before the experiments, reduced food-evoked histamine mobilization by more than 50% (see also Kitano *et al.* 2000*b*) as compared with the intact side of the stomach. In view of the observation that much of the histamine response to electrical vagal stimulation depends on the release of gastrin (see above), the poor histamine response to food intake after vagotomy may well reflect impaired gastrin mobilization (see e.g. Debas & Carvajal, 1994). However, if food-evoked histamine mobilization following vagotomy was reduced because less gastrin was mobilized, it is difficult to explain why unilateral vagotomy reduced the histamine response only on the vagotomized side of the stomach (Fig. 3). Perturbations in the serum gastrin concentration should affect ECL cells on either side of the stomach similarly and simultaneously; moreover, unilateral vagotomy did not affect circulating gastrin levels in either fasted or refed rats (Table 1).

While food-evoked histamine mobilization was reduced by 50% after vagotomy (Fig. 3), the histamine response to near-maximally effective doses of gastrin was unaffected (Fig. 4*A*). This discrepancy may be explained by our observation that unilateral vagotomy caused a shift to the right of the gastrin dose–histamine response curve and the serum gastrin concentration–histamine response curve (Fig. 4*B* and *C*). This was manifested in a reduced response to gastrin (-60%, $P < 0.05$) at circulating gastrin concentrations between 30 and 550 pmol l^{-1} (Fig. 4*C*). Hence, the reduced sensitivity of

the ECL cells to gastrin after vagotomy is manifested only at serum gastrin concentrations within the physiological (and not the supra-physiological) range. While food intake raised the serum gastrin concentration from 16 pmol l^{-1} to 41 pmol l^{-1} (Table 1), intravenous infusion of a near-maximally effective dose of gastrin resulted in circulating gastrin concentrations of more than 1000 pmol l^{-1}. The desensitization of the gastrin receptor after vagotomy was reflected by a 7-fold increase in the gastrin EC_{50} value. Previously, in vagally intact rats, it was noted that the gastrin EC_{50} value was 3- to 4-fold higher in hypergastrinaemic rats than in hypogastrinaemic rats (Konagaya *et al.* 2001). Hence, upon sustained exposure to high concentrations of gastrin the gastrin receptor of the ECL cells changes from a high affinity to a low affinity state. However, to the best of our knowledge, the present finding is the first to suggest that also the vagus is capable of controlling the sensitivity of the gastrin receptor.

Gastrin release may explain why electrical vagal stimulation induces histamine mobilization. However, the response to unilateral vagal stimulation was 2-fold greater on the stimulated than on the non-stimulated side (Fig. 2*C*). This suggests that factors other than circulating gastrin affect the response of the ECL cells. Since vagal denervation was found to shift the serum gastrin concentration–histamine response curve to the right, it is tempting to speculate that vagal stimulation may shift the concentration–response curve in the opposite direction, thereby explaining a greater gastrin-evoked histamine response on the stimulated than on the non-stimulated side.

Histamine mobilization following sympathetic denervation

There is experimental support for the view that sympathetic nerve stimulation may activate the ECL cells directly as well as indirectly: the sympathetic transmitters noradrenaline and adrenaline are capable of acting directly on β-adrenergic receptors on the ECL cells (Lindström *et al.* 1997; Lindström & Håkanson, 2001), but there is also evidence suggesting that they may activate ECL cells *in situ* in an indirect manner (through vasoconstriction-evoked ischaemia) (Norlén *et al.* 2001; Bernsand *et al.* 2003). From the present findings it seems that the sympathetic nervous system plays a minor role (if any) in the food-evoked histamine response: surgical sympathectomy had no impact on either basal, gastrin- or food-evoked histamine mobilization (Fig. 5).

Concluding remarks

We obtained no evidence that acute vagal stimulation induced histamine mobilization by a direct action on the ECL cells. Nonetheless, ablation of the vagal input to the stomach impaired the histamine response of the ECL cells to both food and gastrin. From earlier work, it is known that unilateral vagal denervation leads to a diminishing ECL-cell population on the denervated side (Håkanson *et al.* 1984, 1992; Axelson *et al.* 1988), an observation that has been interpreted to mean that the ECL cells operate under long-term tonic control by the vagus. Here we report that unilateral vagotomy results in a rightward shift of the serum gastrin concentration–histamine response curve on the denervated side of the stomach. This observation is in line with the view that the vagus controls the ECL cell activity and that the suppressed responsiveness of the ECL cells to gastrin after vagotomy reflects gastrin receptor desensitization. Ablation of the sympathetic innervation of the stomach, on the other hand, was without effect on the ECL cells

References

Alino SF, Garcia D & Uvnas-Moberg K (1983). On the interaction between intragastric pH and electrical vagal stimulation in causing gastric acid secretion and intraluminal release of gastrin and somatostatin in anesthetized rats. *Acta Physiol Scand* **117**, 491–495.

Alumets J, Ekelund M, Håkanson R, Hedenbro J, Rehfeld JF, Sundler F & Vallgren S (1982). Gastric acid response to pylorus ligation: Is gastrin or histamine involved? *J Physiol* **323**, 145–156.

Axelson J, Ekelund M, Håkanson R & Sundler F (1988). Gastrin and the vagus interact in the trophic control of the rat oxyntic mucosa. *Regul Pept* **22**, 237–243.

Bernsand M, Ericsson P, Björkqvist M, Zhao CM, Håkanson R & Norlén P (2003). Submucosal microinfusion of endothelin and adrenaline mobilizes ECL-cell histamine in rat stomach, and causes mucosal damage: a microdialysis study. *Br J Pharmacol* **140**, 707–717.

Brodie DA & Knapp PG (1966). The mechanism of the inhibition of gastric secretion produced by esophageal ligation in the pylorus-ligated rat. *Gastroenterology* **50**, 787–795.

Clark JA Jr, Myers PH, Goelz MF, Thigpen JE & Forsythe DB (1997). Pica behaviour associated with buprenorphine administration in the rat. *Laboratory Anim Sci* **47**, 300–303.

Cui GL, Sandvik AK, Munkvold B & Waldum HL (2002). Effects of anaesthetic agents on gastrin-stimulated and histamine-stimulated gastric acid secretion in the totally isolated vascularly perfused rat stomach. *Scand J Gastroenterol* **37**, 750–753.

Debas HT & Carvajal SH (1994). Vagal regulation of acid secretion and gastrin release. *Yale J Biol Med* **67**, 145–151.

Debas HT, Hollinshead J, Seal A, Soon-Shiong P & Walsh JH (1984). Vagal control of gastrin release in the dog: pathways of stimulation and inhibition. *Gastroenterology* **95**, 34–37.

Ding X-Q & Håkanson R (1996). Effect of cholecystokinin-B/gastrin receptor blockade on gastric acid secretion in conscious rats. *Pharmacol Toxicol* **79**, 324–330.

Ekblad E, Ekelund M, Graffner H, Håkanson R & Sundler F (1985). Peptide-containing nerve fibers in the stomach wall of rat and mouse. *Gastroenterology* **89**, 73–85.

Ekblad E, Håkanson R & Sundler F (1991). Innervation of the stomach of rat and man with special reference to the endocrine cells. In *The Stomach as an Endocrine Organ*, ed. Håkanson R & Sundler F, pp. 79–95. Elsevier, Amsterdam.

Ekelund M, Håkanson R, Hedenbro J, Liedberg G, Lundquist I, Rehfeld JF & Sundler F (1982). Effects of insulin on serum gastrin concentrations, gastric acid secretion and histamine mobilization in the rat. *Acta Physiol Scand* **114**, 17–29.

Ekelund M, Håkanson R & Vallgren S (1987). Effects of cimetidine, atropine and pirenzepine on basal and stimulated gastric acid secretion in the rat. *Eur J Pharmacol* **138**, 225–232.

Ericsson P, Norlén P, Bernsand M, Alm P, Höglund P & Håkanson R (2003). ECL cell histamine mobilization studied by gastric submucosal microdialysis in awake rats: Methodological considerations. *Pharmacol Toxicol* **93**, 57–65.

Furness JB, Bornstein JC, Murphy R & Pompolo S (1992). Roles of peptides in transmission in the enteric nervous system. *Trends Neurosci* **15**, 66–71.

Gershon MD, Kirchgessner AL & Wade PR (1994). Functional anatomy of the enteric nervous system. In *Physiology of the Gastrointestinal Tract*, 3rd edn, ed. Johnson LR, vol. 2, pp. 381–422. Raven Press, New York.

Green T & Dockray GJ (1988). Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. *Neuroscience* **25**, 181–193.

Håkanson R, Axelson J, Tielemans Y, Johansson A-G, Willems G & Sundler F (1992). Unilateral vagal denervation suppresses omeprazole-induced trophic effects on the denervated side of the rat stomach. *Scand J Gastroenterol* **27**, 65–70.

Håkanson R, Böttcher G, Ekblad E, Panula P, Simonsen M, Dohlsten M, Hallberg T & Sundler F (1986). Histamine in endocrine cells in the stomach. A survey of several species using a panel of histamine antibodies. *Histochemistry* **86**, 5–17.

Håkanson R, Chen D & Sundler F (1994). The ECL cells. In *Physiology of the Gastrointestinal Tract*, 3rd edn, ed. Johnson LR, vol. 2, pp. 1171–1184. Raven Press, New York.

Håkanson R, Hedenbro J, Liedberg G, Sundler F & Vallgren S (1980). Mechanisms of gastric acid secretion after pylorus and oesophagus ligation in the rat. *J Physiol* **305**, 139–149.

Håkanson R, Hedenbro J, Liedberg G & Vallgren S (1982). Effect of vagotomy on gastric acid secretion in the rat. *Acta Physiol Scand* **115**, 135–139.

Håkanson R, Kroesen JH, Liedberg G, Oscarsson J, Rehfeld JF & Stadil F (1974). Correlation between serum gastrin concentration and rat stomach histidine decarboxylase activity. *J Physiol* **243**, 483–498.

Håkanson R & Sundler F (1991). Histamine-producing cells in the stomach and their role in the regulation of acid secretion. *Scand J Gastroenterol* **26**, 88–94.

Håkanson R, Vallgren S, Ekelund M, Rehfeld JF & Sundler F (1984). The vagus exerts trophic control of the stomach in the rat. *Gastroenterology* **86**, 28–32.

Hannibal J, Ekblad E, Mulder H, Sundler F & Fahrenkrug J (1998). Pituitary adenylate cyclase activating polypeptide (PACAP) in the gastrointestinal tract of the rat: distribution and effects of capsaicin denervation. *Cell Tissue Res* **291**, 65–79.

Holmes TW Jr, Gilfillan RS & Cuthbertson EM (1967). Sympathectomy in the release of vasoconstriction: similarities of response in cerebral palsy and poliomyelitis. *Surgery* **61**, 129–135.

Kitano M, Norlén P, Ding XQ, Nakamura S & Håkanson R (2000*a*). Long-lasting cholecystokinin-2 receptor blockade after a single subcutaneous injection of YF476 or YM022. *Br J Pharmacol* **130**, 699–705.

Kitano M, Norlén P & Håkanson R (2000*b*). Gastric submucosal microdialysis: a method to study gastrin- and food-evoked mobilization of ECL-cell histamine in conscious rats. *Regul Pept* **86**, 113–124.

Konagaya T, Bernsand M, Norlén P & Håkanson R (2001). Mobilization of rat stomach ECL-cell histamine in response to short- and long-term treatment with omeprazole and/or YF 476 studied by gastric submucosal microdialysis in conscious rats. *Br J Pharmacol* **133**, 37–42.

Lawton GP, Tang LH, Miu K, Gilligan CJ, Absood A & Modlin IM (1995). Adrenergic and cromolyn sodium modulation of ECL cell histamine secretion. *J Surg Res* **58**, 96–104.

Lindström E, Björkqvist M, Boketoft Å, Chen D, Zhao CM, Kimura K & Håkanson R (1997). Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. *Regul Pept* **71**, 73–86.

Lindström E & Håkanson R (2001). Neurohormonal regulation of secretion from isolated rat stomach ECL cells: a critical reappraisal. *Regul Pept* **97**, 169–180.

Morley JS & Smith JM (1968). Polypeptides. IX. Variations of the methionyl position in the C-terminal tetrapeptide amide sequence of the gastrins. *J Chem Soc* **6**, 726–733.

Morley JS, Tracy HJ & Gregory RA (1965). Structure-function relationships in the active C-terminal tetrapeptide sequence of gastrin. *Nature* **207**, 1356–1359.

Nishida A, Uchida-Kobayashi A, Takemoto Y, Akuzawa S & Miyata K (1996). Comparative evaluation of the role of endogenous gastrin in basal acid secretion in conscious rats provided with chronic fistula and pylorus ligation. *Jpn J Pharmacol* **71**, 223–230.

Nishimura E, Buchanan AM & McIntosh CH (1984). Autoradiographic localization of opioid receptors in the rat stomach. *Neurosci Lett* **50**, 73–78.

Norlén P, Bernsand M, Konagaya T & Håkanson R (2001). ECL-cell histamine mobilization in conscious rats: Effects of locally applied regulatory peptides, candidate neurotransmitters and inflammatory mediators. *Br J Pharmacol* **134**, 1767–1777.

Norlén P, Kitano M, Lindström E & Håkanson R (2000). Anaesthetic agents inhibit gastrin-stimulated but not basal histamine release from rat stomach ECL cells. *Br J Pharmacol* **130**, 725–730.

Prinz C, Kajimura M, Scott DR, Mercier F, Helander HF & Sachs G (1993). Histamine secretion from rat enterochromaffinlike cells. *Gastroenterology* **105**, 449–461.

Riedel R, Bohnenkamp W, Eltze M, Heintze K, Prinz W & Kromer W (1988). Comparison of the gastric antisecretory and antiulcer potencies of telenzepine, pirenzepine, ranitidine and cimetidine in the rat. *Digestion* **40**, 25–32.

Sandor A, Kidd M, Lawton GP, Miu K, Tang LH & Modlin IM (1996). Neurohormonal modulation of rat enterochromaffin-like cell histamine secretion. *Gastroenterology* **100**, 1084–1092.

Sandvik AK, Waldum HL, Kleveland PM & Schulze-Søgnen B (1987). Gastrin produces an immediate and dose-dependent histamine release preceeding acid secretion in the totally isolated, vascularly perfused rat stomach. *Scand J Gastroenterol* **22**, 803–808.

Schubert ML, Bitar KN & Makhlouf GM (1982). Regulation of gastrin and somatostatin secretion by cholinergic and non-cholinergic intramural neurons. *Am J Physiol* **243**, G442–447.

Schultzberger M, Hökfelt T, Nilsson G, Terenius L, Rehfeld JF, Brown M, Elde R, Goldstein M & Said S (1980). Distribution of peptide- and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal peptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine β-hydroxylase. *Neuroscience* **5**, 689–744.

Sternini C, Patierno S, Selmer IS & Kirchgessner A (2004). The opioid system in the gastrointestinal tract. *Neurogastroenterol Motil* **16** (Suppl. 2), 3–16.

Thompson AC, Kristal MB, Sallaj A, Acheson A, Martin LB & Martin T (2004). Analgesic efficacy of orally administered buprenorphine in rats: methodological considerations. *Comp Med* **54**, 293–300.

Vallgren S, Ekelund M & Håkanson R (1983). Mechanism of inhibition of gastric acid secretion by vagal denervation in the rat. *Acta Physiol Scand* **119**, 77–80.

Waldum HL, Sandvik AK, Brenna E & Petersen H (1991). The gastrin-histamine sequence in the regulation of gastric acid secretion. *Gut* **32**, 698–700.

Zeng N, Athman C, Kang T, Lyu R-M, Walsh JH, Ohning GV, Sachs G & Pisegna JR (1999). PACAP type 1 receptor activation regulates ECL cells and gastric acid secretion. *J Clin Invest* **104**, 1383–1391.

Zeng N, Kang T, Lyu R-M, Wong H, Wen Y, Walsh JH, Sachs G & Pisegna JR (1998). The pituitary adenylate cyclase activating polypeptide type 1 receptor (PAC_1-R) is expressed on gastric ECL cells: Evidence by immunocytochemistry and RT-PCR. *Ann NY Acad Sci* **865**, 147–156.

Zhao CM, Chen D, Monstein HJ, Ding XQ, Sundler F & Håkanson R (1996). Rat stomach enterochromaffin-like cells are not stimulated by pylorus ligation. A biochemical and ultrastructural study. *Scand J Gastroenterol* **31**, 31–37.

Acknowledgements

We are grateful to Dr P. Hedlund (Lund) for sharing his expertise on electrophysiology. This study was supported by grants from the Swedish Research Council (04X-01007), Påhlsson's Foundation, the Åke Wiberg foundation, the Magnus Bergvall foundation, the Novo-Nordisk Foundation, Nio Meter Liv, the Swedish Society of Medical Science, the Juhlin Foundation, the Crafoord Foundation, Svenska Läkaresällskapet, Kungliga Fysiografiska Sällskapet of Lund, and the Medical Faculty of Lund University.